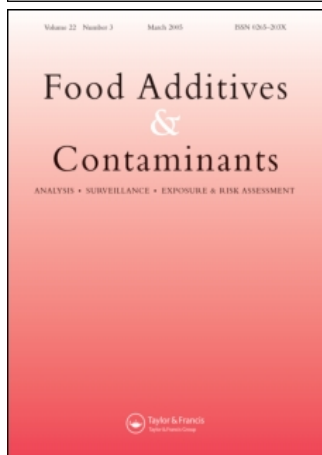


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Population ecology of *Aspergillus flavus* associated with Mississippi Delta soils

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Abstract

Understanding the source of *Aspergillus flavus* is required to manage aflatoxin contamination of maize (*Zea mays* L.). Studies assessed *A. flavus* propagules, *Fusarium* spp., and total fungi associated with Mississippi Delta soils. Soils from 12 and 15 sites were collected in 2000 and 2001, respectively. The propagule density of *A. flavus* ranged from log(10) 2.0 to 4.3 colony-forming units (cfu) g⁻¹ soil, while total fusaria ranged from log(10) 3.0 to 5.4 cfu g⁻¹ soil. The highest populations of *A. flavus* were associated with soils containing higher organic matter, especially in sites under a no-tillage management. The frequency of aflatoxin production in isolates ranged from 13 to 81% depending on soil. In 2001, there was a highly significant correlation between *A. flavus* and the history of maize cultivation. Soil fertility factors such as organic matter content, nitrate and extractable phosphorus correlated with the density of *Aspergillus*, *Fusarium* spp., and total fungi. The relationship between soil parameters and *Aspergillus* populations may be useful in predicting the contribution of soil microflora to aflatoxin contamination.

Keywords: Aflatoxin, *Aspergillus flavus*, *Fusarium* spp, maize (corn, *Zea mays* L.), soil ecology

Introduction

The fungus *Aspergillus flavus* (Link) is ubiquitous in tropical/subtropical environments (Klich 2002a) and is capable of colonizing many important crops such as maize (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), peanuts (*Arachis hypogea* L.), and tree nuts resulting in the accumulation of aflatoxins. When levels of aflatoxin are high enough to impact human or animal health negatively, food producers experience significant economic damage because of reduced demand or crop damage. In order to manage aflatoxin contamination in the field effectively, it is necessary to understand the ecology and epidemiology of *A. flavus* (Wicklow et al. 1998; Horn 2003).

The ecology of soil populations of *A. flavus* has been characterized for maize production systems in the Midwestern USA (McGee et al. 1996; Wicklow et al. 1998), for peanut soils in the Southern USA

(Griffin et al. 1981; Horn and Dorner 1998), and for Arizona and Texas cotton soils in the Southwestern USA (Orum et al. 1997; Jamie-Garcia and Cotty 2006). Overall populations of *A. flavus* associated with the Southern USA are much higher than those observed in the Midwestern USA, which are primarily attributed to a warmer soil temperatures further south. Most of the more productive soils in the Mississippi Delta region of the USA were traditionally planted to cotton. However, in recent years there has been a diversification of this monoculture to include maize in a rotation to improve yields, economic returns, reduce pests, and improve soil quality (Reddy et al. 2006). The present study was conducted to assess *A. flavus* populations associated with soils and cropping systems of the Mississippi Delta region of the Southern USA and to gain insights into the contribution of soil properties toward modulating the propagule density of *A. flavus*.

Materials and methods

Soils

Surface soils (0–5 cm) were collected from experimental sites and producer farms in Leflore, Washington, and Sunflower counties of Mississippi. Twelve and 15 sites were sampled in 2000 and 2001, respectively. Many of the sites were sampled as part of the Mississippi Delta Management Evaluation Study Area Project (Locke 2004) designed to understand the relationship between management practices and the quality of soil and water resources. In this study most sites were sampled in both years. Soils classified by soil surveys were mostly a Dundee series (fine-silty, mixed, active, thermic Typic Endoaqualfs); and soils in the Forestdale (fine, smectitic, thermic Typic Endoaqualfs), Dowling (very fine smectitic, non-acid, thermic Vertic Endoaqualfs), and Alligator (very fine, smectitic thermic Chromic Dystraquerts) soil series were also among soils sampled (USDA-NRCS 2006).

The soils were collected in early to mid-March before planting and the application of herbicide and fertilizer. Sampling points were geo-referenced with a global positioning system (GPS) to enable precise resampling. At all sites the cropping histories (corn production) for at least the past 10 years before sample collection were obtained from farm managers. Soils were collected from both conventional tillage (CT) and no tillage (NT) systems. At each site four replicate samples were collected approximately 100 m apart in a rectangular grid using a surface-sterilized sampling probe (of 5 cm diameter). All replicate samples for individual sites were collected within the same soil series and landscape position. Each replicate soil sample consisted of a composite of ten subsamples collected to a depth of 0–5 cm. Soils were sieved to pass a surface-sterilized 2.25-mm mesh screen to remove plant debris and moisture content determined gravimetrically. Soils were maintained at 4°C until fungal propagules were enumerated within 2 days of collection.

Microbial characteristics

Aspergillus propagules were enumerated on modified dichloronitroaniline Rose Bengal (MDRB) media as described by Horn and Dorner (1998). Soils (2.5 or 0.5 g) were suspended in 10 ml of dilute (0.2%) water agar, 250 µl of each dilution were spread on duplicate plates, and were incubated at 37°C for 72 h before counting. Only colonies exhibiting typical *A. flavus* morphology (Klich 2002b) were counted. From each replicate site four to six colonies were randomly selected and transferred to potato dextrose

agar for assessment of aflatoxin production based on an ELISA assay (Abbas et al. 2004a). Total fungal propagules and *Fusarium* propagules were determined by serial dilution plating on rose bengal potato dextrose agar (Martin 1950) and peptone-pentachloronitrobenzene (PCNB) media (Nash and Snyder 1962), respectively, as described elsewhere except dilutions (50 µl), which were dispensed and spread using a spiral plater (Spiral Systems, Bethesda, MD, USA). Rose bengal potato dextrose agar and peptone-PCNB plates were counted 5 and 4 days after plating, respectively. Microbial propagule densities (colony-forming units, cfu) were calculated on a soil dry weight basis and transformed on a log(10) scale.

Soil characteristics

Soil moisture content was calculated gravimetrically following drying at 70°C for 72 h. Soil analysis was conducted on air dried soil. Soil texture (sand, silt, and clay) was determined by the hydrometer method (Gee and Bauder 1986). Air-dried soils were analysed by the University of Arkansas Soil Analysis Laboratory (Marianna, AR, USA) for nutrients. Organic matter was assessed by loss of mass after ignition (Nelson and Sommers 1996). Phosphate and potassium were determined by plasma emission spectroscopy in Mehlich 3 soil extracts (Mehlich 1984). Nitrate was extracted in 0.25 M aluminium sulfate and was determined by specific ion electrode (Donahue 1992). Electrical conductivity (EC) and pH were determined in an aqueous soil suspension (2:1).

Statistical analysis

Estimates of microbial propagule density were analysed by analysis of variance and mean separation by Fisher LSD using Proc GLM of the SAS Institute (2001). Pearson correlations were used to assess the relationship between *A. flavus*, *Fusarium* spp., and total fungal propagule densities, and the site variables of maize history and soil properties (organic matter, pH, electrical conductivity, NO₃, P, K, sand, silt and clay) using Proc Corr of SAS.

Results and discussion

Chemical and physical properties of soils

The ranges of characteristics of soil sampled in both years are summarized in Table I. Specific data for some chemical and physical properties of most soils are presented elsewhere (Zablotowicz et al. 2006). There was a wide range of organic matter content in these soils (0.7–4.8%), and the highest organic matter was typically associated with soils under

Table I. Simple statistics of microbial propagules and properties of soils evaluated in 2000 and 2001.

Variable	Mean \pm standard deviation		Minimum		Maximum	
	2000	2001	2000	2001	2000	2001
Maize history (years in the last 10 years)	1.3 \pm 0.8	1.9 \pm 1.1	0	3	0	4
<i>Aspergillus flavus</i> (log 10 cfu g ⁻¹)	3.2 \pm 0.6	2.6 \pm 0.6	2.2	1.9	4.4	4.2
<i>Fusarium</i> sp. (log 10 cfu g ⁻¹)	3.9 \pm 0.5	3.4 \pm 0.5	3.0	2.6	5.6	4.7
Total fungi (log 10 cfu g ⁻¹)	5.4 \pm 0.4	5.2 \pm 0.5	4.7	4.3	6.3	6.8
Moisture content (%)	14.1 \pm 3.8	15.9 \pm 3.8	6.2	9.9	24.8	24.3
Organic matter (%)	1.5 \pm 0.9	1.9 \pm 0.9	0.7	0.8	4.3	4.8
pH	6.4 \pm 0.6	6.3 \pm 0.4	5.2	5.6	7.5	7.1
Electrical conductivity (μ S cm ⁻¹)	133 \pm 122	52 \pm 11	48	17	550	193
NO ₃ -N (mg kg ⁻¹)	20 \pm 34	5.7 \pm 4	2	2.0	144	24
P (mg kg ⁻¹)	62 \pm 52	60 \pm 42	9	10	233	206
K (mg kg ⁻¹)	425 \pm 213	312 \pm 84	184	116	1051	489
Sand (%)	26 \pm 7	24 \pm 10	3	3	49	49
Silt (%)	52 \pm 13	50 \pm 12	22	22	80	80
Clay (%)	21 \pm 7	25 \pm 10	13	12	43	48

no-tillage management. Soil pH ranged from slightly acidic (5.2) to slightly alkaline (7.5). Based on particle size distribution, most soils assessed ranged from a silt loam to silty clay loam textural classification. These soils exhibited a wide range of fertility, which is evident by range of potassium, nitrate, and phosphorous concentrations. Electrical conductivity is a measurement of solutes in soil solution and is also associated with aspects of fertility, e.g. soluble nitrogen and potassium. The highest nutrient levels were found in the soils from the DW3 site that was under no tillage for several years and was amended with various organic wastes (poultry litter and cotton gin extrusions) and also had the highest organic matter content.

Aspergillus flavus and other microbial populations in soils

In 2000 samples, estimates of *A. flavus* propagule density in soils collected from 12 sites ranged from log(10) 2.3 to 4.3 cfu g⁻¹ soil (Table II). By comparison, propagule densities of *Fusarium* spp. ranged from log(10) 3.3 to 5.4 cfu g⁻¹ soil, while total culturable fungal propagules enumerated ranged from log(10) 4.8 to 6.2 cfu g⁻¹ soil using cultural methods on a semi-selective and non-selective media, respectively. Based on these estimates, the populations of *A. flavus* typically ranged from less than 0.1 to about 8% of the total fungal population, while total *Fusarium* spp. were typically more numerous ranging from about 1.1 to 6.6% of the total culturable fungal population. Populations of all microorganisms enumerated were highest in soils with the greatest organic matter content. This becomes more obvious when comparing the high organic matter soil DW1a vs. the

low organic matter soil DW1b from the same field; DW2, a conventionally tilled soil adjacent to the no-tillage soil with additional organic matter added (soil DW3); and the comparison of Stoneville NT vs. Stoneville CT soils. Overall, aflatoxigenic isolates recovered from the various soils ranged from about 31 to 75% with a similar range found in soils with no maize history as soils with a history of maize cultivation. Due to limitations in resources, it was not possible to characterize a larger sample of individual isolates from each soil. Therefore, valid statistical comparisons of aflatoxigenic isolates frequency among the various soils were not attempted.

In soil samples in 2001, estimates of *A. flavus* enumerated from 15 soils ranged from 2.0 to 3.8 log(10) cfu g⁻¹ of soil, while *Fusarium* spp. ranged from 3.0 to 4.7 log(10) cfu g⁻¹ of soil (Table III). Based on these estimates, the populations of *A. flavus* represented from <0.1 to 2.6% of the total fungal population, while *Fusarium* spp. populations ranged from about 0.8 to 4.0% of the total fungal population. Populations of *A. flavus* enumerated from these soils were consistent to those reported for soils from warm temperate to semi tropical climates used for peanut, corn, and cotton production (Horn and Dorner 1998; Jamie-Garcia and Cotty 2006). Populations of *Fusarium* spp. enumerated in Mississippi soils were up to ten-fold higher than those reported elsewhere (Nash and Snyder 1962; Nemec et al. 1989). However, propagules were examined the surface 0–5 cm in this current study, and organic matter content was much higher than that in the sandy Florida soils (Nemec et al. 1989). The mean propagule density for *A. flavus* in 2001 was log(10) 2.60, much lower

Table II. Propagule density of *Aspergillus flavus* and other soil microflora enumerated from 12 Mississippi Delta soils sampled before planting in 2000.

Site/maize history	Propagule density (log 10 cfu g ⁻¹ soil) ¹			Percentage of total fungi		<i>A. flavus</i> assayed for aflatoxin production	
	<i>A. flavus</i>	<i>Fusarium</i> spp.	Total fungi	<i>A. flavus</i>	<i>Fusarium</i> spp.	Number of isolates tested ²	Aflatoxin positive
<i>Maize history</i>							
Beasley 1 (CT)	3.5 d ³	4.0 b	5.8 b	0.5	1.5	16	11
Beasley 2 (CT)	3.7 c	3.8 b,c	5.5 c	1.5	2.0	15	9
DW 1a (NT)	4.0 b	4.0 b	5.1 d	8.1	6.6	16	12
DW 1b (NT)	3.7 c,d	3.3 e	4.8 e	7.5	3.3	16	10
DW2 (CT)	2.9 g	3.6 c,d	5.5 c	0.2	1.1	27	16
DW3 (NT)	4.3 a	5.4 a	6.2 a	0.7	3.3	16	9
GW-L (CT)	3.0 f,g	3.5 d,e	5.1 d	0.8	2.3	16	5
GW-Mon (CT)	2.3 i	4.0 b	5.4 c	<0.1	3.7	8	3
Indian Mound (CT)	3.3 e	3.7 b,c,d	5.4 c	0.8	2.0	15	10
<i>No maize</i>							
Beasley 3 (CT)	2.4 i	3.6 c,d	5.1 d	0.2	3.3	10	5
Stoneville (CT)	2.6 h	3.6 c,d	5.2 d	0.3	1.3	11	4
Stoneville (NT)	3.1 f	3.9 b	5.5 c	0.4	3.7	16	10
LSD 0.05	0.2	0.3	0.2				

¹Mean of four replicate samples per site.²Aflatoxin production determined by ELISA was typically assessed on four individual colonies isolated from each of the four replicate soil samples.³Means of population estimates followed by the same letter do not differ significantly at the 95% confidence level using Fisher's LSD test. CT, conventional tillage; NT, no tillage.Table III. Propagule density of *Aspergillus flavus*, *Fusarium* spp. and other soil microflora enumerated from 15 Mississippi Delta soils sampled before planting in 2001.

Site/maize history	Propagule density (log 10 cfu g ⁻¹ soil) ¹			Percentage of total fungi		<i>A. flavus</i> assayed for aflatoxin production	
	<i>A. flavus</i>	<i>Fusarium</i> spp.	Total fungi	<i>A. flavus</i>	<i>Fusarium</i> spp.	Number of isolates tested ²	Aflatoxin positive
<i>Maize history</i>							
Beasley 1 (CT)	3.0 c,d ²	3.9 b	5.9 b	0.6	4.9	16	2
Beasley 2 (CT)	2.0 i	3.0 e	4.9 i	0.1	1.0	16	10
DW 1a (NT)	3.4 b	3.3 c,d	5.0 g,h,i	2.5	1.9	16	8
DW 1b (NT)	2.3 g,h,i	3.1 d,e	4.9 h,i	0.2	2.5	16	9
DW2 (CT)	3.0 c,d	3.1 d,e	4.5 j	2.6	4.0	16	9
DW3 (CT)	3.8 a	4.6 a	6.7 a	0.1	1.0	16	13
Elizabeth (CT)	2.4 f,g,h	3.0 e	5.1 e,f	0.2	0.8	16	5
GW-L (CT)	2.2 g,h,i	3.4 c	5.1 f	0.1	1.9	20	9
GW-Mon (CT)	2.0 i	3.1 d,e	4.9 h,i	0.1	1.5	11	3
Indian Mound (CT)	3.0 c	3.4 c	5.4 d	0.1	1.2	20	7
Thighman 1 (NT)	2.5 e,f,g	3.5 c	5.4 d	0.1	1.2	16	8
Thighman 2 (NT)	2.8 c,d,e	3.1 d,e	4.8 i	1.0	2.0	16	8
Thighman 4 (CT)	2.7 d,e,f	3.9 b	5.6 c	0.1	2.0	16	10
<i>No maize</i>							
Beasley 3 (CT)	2.0 i	3.1 d,e	5.1 e,f	<0.1	1.1	26	17
Thighman 3 (CT)	2.0 i	3.5 c	5.3 d,e	<0.1	1.8	14	7

¹Log 10 colony-forming unit g⁻¹ soil, mean of four replicate samples per site, means followed by the same letter do not differ significantly at the 95% confidence level using Fisher's LSD test.²Aflatoxin production determined by ELISA was typically assessed on four individual colonies isolated from each of the four replicate soil samples.³Means of population estimates followed by the same letter do not differ significantly at the 95% confidence level using Fisher's LSD test. CT, conventional tillage; NT, no tillage.

than the mean of 3.23 cfu g^{-1} soil in 2000. Box plots were used to illustrate the distribution and skewedness of population estimates for the two fungal species and total culturable fungal propagules (Figure 1). In both years the median and mean of *A. flavus* populations were nearly identical, while the mean was typically greater than the median for *Fusarium* spp. and total fungi in 2001. Most of the upper outlier points were from the GW3 soil that was very rich in organic matter. These differences in *A. flavus* and fungal populations in these 2 years may be due to differences in the weather patterns. For example, the 3 months preceding sampling in 2001 were colder and wetter than in 2000 (Table IV). In addition, there were slightly higher moisture contents in most soils sampled in 2001, agreeing with increased rainfall (Table I). The frequency of

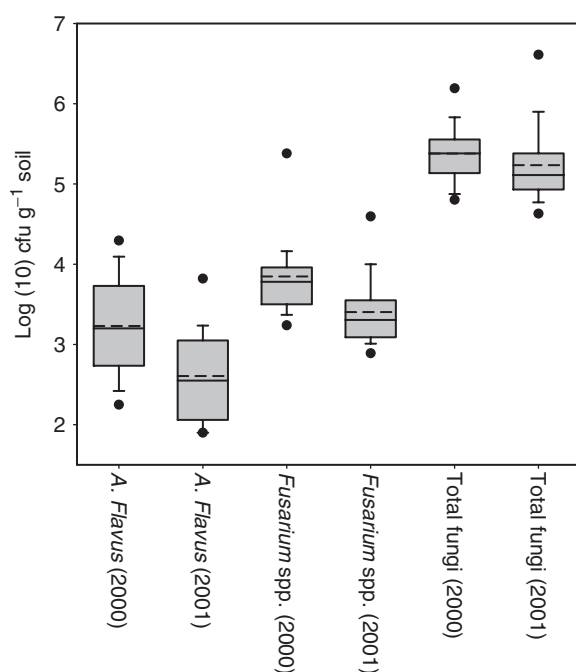


Figure 1. Box plots of fungal populations enumerated from Mississippi Delta soils in 2000 and 2001. The box illustrates the 25 and 75% percentiles with the solid and dashed lines being the median and mean, respectively. Whisker bars indicate the 10 and 90 percentiles; circles the 5 and 95% outlier points.

Table IV. Average climatic conditions January–March in the 2 years of the survey.

Parameter ¹	2000	2001
Air maximum temperature (°C)	16	12
Air minimum temperature (°C)	5	4
Soil maximum temperature 5 cm (°C)	15	12
Soil minimum temperature 5 cm (°C)	12	7
Average precipitation (cm day ⁻¹)	0.17	0.20

¹Weather data were compiled from the Mississippi State University weather site for the Beasley Lake site.

aflatoxigenic isolates (44% in soils with maize history compared with 58% in soils never cultivated with maize) was within a similar range as those observed in 2000. Overall greater than 80% of the aflatoxigenic isolates recovered from soil produced $>1000 \text{ ng g}^{-1}$ fresh weight of fungal biomass (data not shown).

Correlations of microbial populations and soil properties

In soils collected in 2000, there was no correlation between maize cultivation and any of the four microbial populations (Table V). However, in the soils collected in 2001 the populations of *A. flavus* were highly correlated with years of maize cultivation. In 2001 there were more soils evaluated and the range of maize history was from zero to 4 years in the previous decade. Typically, all three groups of fungi were more numerous with increased fertility. The higher population densities were associated with soils of high organic matter, abundant nitrate, phosphate, and potassium. An example of the correlation of *A. flavus* propagules enumerated from 12 soils in 2000 vs. soil organic matter content is presented in Figure 2. Likewise, the more abundant populations were associated with increased pH and higher electrical conductivity (EC).

The highest populations of *A. flavus* were found in soils following maize in a study conducted in South Texas (Jamie-Garcia and Cotty 2006) and in a growers field in Mississippi (Abbas et al. 2004b). The significant correlation between organic matter content and *A. flavus* propagules was consistent with reports by Wicklow et al. (1984) and Klich (2000a) who found that *Aspergillus* propagules survive over-wintering as sclerotia associated with crop debris. Adoption of no-tillage practices

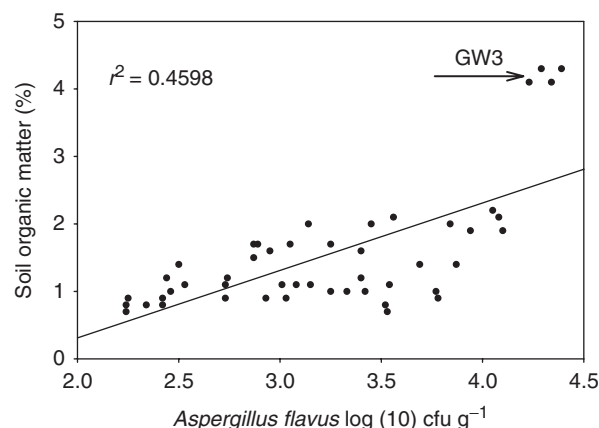


Figure 2. Correlation of *Aspergillus flavus* populations with soil organic matter content in soils collected in 2000. All four soils from the high organic management site GW3 had twice the organic matter content of most soils and the highest *A. flavus* propagules.

Table V. Pearson correlations of microbial propagule density with various properties of 12 Mississippi Delta soils sampled in 2000 and 2001, and significance of correlation.

Microbial group	Maize history	Organic matter	Soil pH	EC ¹	Moisture	NO ₃	P	K	Sand	Silt	Clay
<i>2000 soils</i>											
<i>A. flavus</i>	0.099 ²	0.678	0.384	0.371	0.446	0.376	0.545	0.400	0.327	−0.461	0.325
	<i>0.504</i>	<i><0.001</i>	<i>0.007</i>	<i>0.009</i>	<i>0.002</i>	<i>0.009</i>	<i><0.001</i>	<i>0.005</i>	<i>0.023</i>	<i><0.001</i>	<i>0.024</i>
<i>Fusarium</i> spp.	−0.116	0.826	0.460	0.541	0.461	0.625	0.762	0.752	0.008	0.009	−0.048
	<i>0.431</i>	<i><0.001</i>	<i>0.001</i>	<i><0.001</i>	<i>0.001</i>	<i><0.001</i>	<i><0.001</i>	<i><0.001</i>	<i>0.955</i>	<i>0.954</i>	<i>0.747</i>
Total fungi	0.117	0.672	0.334	0.414	0.405	0.573	0.455	0.722	−0.096	−0.027	0.148
	<i>0.429</i>	<i><0.001</i>	<i>0.020</i>	<i>0.003</i>	<i>0.004</i>	<i><0.001</i>	<i>0.001</i>	<i><0.001</i>	<i>0.518</i>	<i>0.855</i>	<i>0.316</i>
<i>2001 soils</i>											
<i>A. flavus</i>	0.412	0.551	0.328	0.446	−0.219	0.384	0.494	0.085	0.082	0.105	−0.178
	<i>0.001</i>	<i><0.001</i>	<i>0.001</i>	<i><0.001</i>	<i>0.093</i>	<i>0.003</i>	<i><0.001</i>	<i>0.516</i>	<i>0.536</i>	<i>0.425</i>	<i>0.173</i>
<i>Fusarium</i> spp.	0.098	0.660	0.005	0.220	0.088	0.609	0.517	0.234	−0.071	−0.048	0.036
	<i>0.454</i>	<i><0.001</i>	<i>0.970</i>	<i>0.091</i>	<i>0.505</i>	<i><0.001</i>	<i><0.001</i>	<i>0.072</i>	<i>0.595</i>	<i>0.717</i>	<i>0.786</i>
Total fungi	0.167	0.623	−0.061	0.173	0.149	0.610	0.460	0.265	−0.083	0.086	−0.003
	<i>0.202</i>	<i><0.001</i>	<i>0.644</i>	<i>0.181</i>	<i>0.250</i>	<i><0.001</i>	<i><0.001</i>	<i>0.041</i>	<i>0.530</i>	<i>0.516</i>	<i>0.985</i>

¹ EC, electrical conductivity.² Correlation upper number, Pr > F in italics.

generally increases soil organic matter in the surface soil (Reeves 1997; Locke et al. 2005). We have observed increased populations of *A. flavus* in no-tillage fields compared with adjacent conventionally tilled soils. However, McGee et al. (1996) found that similar populations of *A. flavus* were observed under conventionally tilled and no-tilled soils. With regard to relationships between crop history and *A. flavus* populations, McGee et al. observed that under conditions in Iowa, USA, *A. flavus* populations were higher following continuous maize than soybeans. Jamie-Garcia and Cotty (2006) observed that soils of South Texas with high clay and a low sand content favoured high aflatoxin contamination in cotton grown in such soils. The incidence of the small sclerotia type, and to a lesser extent *A. flavus* populations (cfu), were positively correlated with clay content. They also found a highly significant correlation between soil clay content and *A. flavus* soil populations in the semi-arid region. Soils with a high clay content have a greater moisture holding potential and this may have provided a greater opportunity for survival of *A. flavus* propagules.

In the present study the highest propagule densities of *A. flavus* were associated with soils having the greatest fertility. Thus, the greatest potential for aflatoxin contamination of maize by soil inoculum of *A. flavus* may be associated with some of the more productive soils. However, crop physiology can impact the severity of aflatoxin contamination, and healthier plants may be less prone to infection. Soils with high organic matter have a greater moisture-holding capacity than soils with a lower organic matter content. Thus, there may be a reduced potential for moisture stress

and susceptibility to aflatoxin contamination in soils with a high organic matter content.

In addition to developing an understanding of soil factors regulating *A. flavus* populations, the present study provided valuable fungal isolates that were used to assess the diversity of *A. flavus* in Mississippi soils (Abbas et al. 2004a, 2005; Baird et al. 2006). A major impact of this study was a source for non-aflatoxigenic strains that have the potential to control aflatoxin in maize (Abbas et al. 2006).

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